In Vitro Antitrypanosomal Activity of *Ocimum gratissimum* Leaf Methanol Extract Against *Trypanosoma brucei brucei*

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Abstract
This study was designed to investigate the antitrypanosomal activity of the leaf methanol extract from Ocimum gratissimum against the pathogenic protozoan Trypanosoma brucei brucei. The leaf methanol extract was prepared by macerating the leaves extracting the macerates in methanol; filtering the extract and evaporating the resulting filtrate to dryness. The antitrypanosomal activity was assessed using a bioassay guided principles and was expressed as percentage growth inhibition. The results showed that O. gratissimum leaf extract obtained from Okene has antitrypanosomal activity as reflected in its IC_{50} 1.02±0.002 µg/ml. This indicates that the methanol extract of O. gratissimum leaf possess antitrypanosomal activity against T. brucei brucei. It is recommended that this plant should be considered and included among ethnomedicinal plants used against trypanosomiasis in Nigeria. It is also recommended that further investigations are needed to determine the active components of this plant extract that are responsible for the observed antitrypanosomal activity.

Keywords: *Trypanosomiasis, Ocimum gratissimum, Antitrypanosomal, Trypanosoma brucei brucei, Methanol extract and IC_{50}*

Introduction

African Trypanosomiasis (AT) also known as sleeping sickness or Nagana is one of the neglected tropical diseases that affect the sub-Sahara African region. It is caused by Trypanosomes a flagellated eukaryotes protozoan (Bizimana *et al.*, 2006). African Trypanosomiasis is transmitted by Tsetse fly of the genus Glossina (Jamonneau *et al.*, 2004). There are two types of African Trypanosomiasis, Human African Trypanosomiasis (HAT) and African Animal Trypanosomiasis (AAT) (Nagana). Human African Trypanosomiasis is caused by *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* because they have the ability to resist apolipoprotein A (Apo L), a protein that can trigger the death of the trypanosomes in humans (Vanhollebeke and Pays, 2010) and African Animal Trypanosomiasis is caused by *Trypanosoma brucei brucei* a sub species

of the *T. brucei* that infects only animals, *Trypanosoma congolense* and *Trypanosoma vivax* (Geiger *et al*, 2018). The virulence of trypanosomes vary in the human immune system this is because of their ability to perform antigenic variation where they change their variant surface glycoproteins in response to production of anti-bodies by the host (Horn, 2014).

Trypanosomiasis has serious draining effect on socioeconomic status and constitute environmental constrain to Livestock production of the sub-Sahara region. Finelle, (1990) and FAO, (2023) reported that about 60 million people are estimated to be possibly vulnerable to HAT in sub-Sahara Africa and about 1.2 billion US dollar is loss due to milk and meat wastage; livestock production in this zone has been limited to about 30 million out of 140 million cattle reared in Africa.

The disease has given rise to situation of medical significance that has attracted attention in search for eradication or control such as the use of Chemotherapeutics like pentamidine, Suramin, Melasoprol, eflorinithine, arsobal and Mel B (Steverding and Tyler, 2005). The use of these chemotherapies has been facing various challenges like toxicity, less effective, expensive and lengthy parenteral administration (Legros et al, 2002) and to some extent drug resistance (Hotez et al, 2007). This has necessitated search for more potent antitrypanosomal compounds to curb the problem of this disease. Nigeria is naturally endowed with diverse vegetations which have potential of ethnomedicinal values. The plant targeted in this work is *Ocimum gratissimum.*

Ocimum gratissimum (*O. gratissimum*) is a plant that belongs to the family Lamiaceae and it is distributed within Africa, South Asia, South America and other tropical regions of the world (Singh, 2012). *O. gratissimum* was reported to contain phytochemicals like, steroids, tannins, flavonoids, saponins, terpenoids alkaloids, inulins, phenolic compounds, B-carotene, glycosides, carotenoids, reducing sugars, phlobatannins, anthraquinones and cardiac glycosides, steroidal ring and deoxy–sugar, quinones, coumarins, and catechins (Akinmoladun *et al*., 2007; Chetia, Upadhyaya and Saikia, 2014). These might have conferred the pharmaceutical uses of been antibacterial, antifungal (Iqbal and Mishra, 2015), antioxidant (Mahapatra *et al*., 2009), and antidiabetic (Egesie, *et al*., 2006; Nwanjo and Oze, 2007). The antitrypanosomal activity of *O. gratissimum* leaf extract obtained from Ogun State, Nigeria was earlier reported (Abiodun *et al.*, 2012). We are currently working to investigate the antitrypanosomal activity of *O. gratissimum* found in other location in Nigeria. This would help us to know if the antitrypanosomal potentials in the plant is not restricted by geographical location and thereby harness the antitrypanosomal potentials in this plant located in other locations apart from the earlier reported locations like Ogun State (Abiodun *et al.*, 2012).

Statement of research problem

Trypanosomiasis has become endemic in Nigeria and other countries of the sub-Sahara. This is greatly affecting the socioeconomic growth of this tropical region. Chemotherapy approach in control and management have faced many challenges like ineffective drugs, cytotoxicity, inability of the affected population to afford the drugs because of lack of resources, development of resistance to drugs by the parasites (Musanabaganwa, 2014; Abubakar, *et al.*, 2019; Ventrelli, *et al.*, 2022).There is a need to search for more potent and novel drug agents that can help alleviate the challenges

Justification

The persistency of trypanosomiasis warrants investigations into finding lasting solution to the problem. The diversity of forest and vegetation in Nigeria provides opportunity of getting natural products that could be antitrypanosomal in the fight to eliminate trypanosomiasis. It is therefore

expedient if these potentials in plant like *O. gratissimum* can be harnessed to save humanity from the scourge of the disease

Objective

The work aims at carrying out in vitro antitrypanosomal activity of *O. gratissimum* leaf methanol extract. The following are the objectives of the work;

1. To investigate in vitro antitrypanosomal activity of *O. gratissimum* leaf methanol extract.
2. To estimate the IC_{50} of *O. gratissimum* leaf methanol extract.

Materials and Method

Chemicals and Reagents

The chemicals and reagent were of analytical grade and obtained from BDH Ltd, UK and Sigma Chemical Company.

Collection and Authentication of *Ocimum gratissimum*

O.gratissimum leaves were collected from Okene, Kogi State. The plant was identified and authenticated by a botanist in Department of Biology, Federal College of Education, Okene. The leaves were dried under shade at room temperature in the laboratories, Department of Integrated Science FCE, Okene.

Preparation of Extract

The dried leaves were pulverized in the laboratory, Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria. The pulverized sample ere weighed, packaged in black poly-ethene bag and stored for solvent extraction.

The method of cold maceration was used for the solvent extraction. 3g of pulverized *O. gratissimum* sample was soaked in 12 Litres of methanol for 72 hours. During the extraction, there was constant stirring of the soaked sample to maximize the extraction efficiently. The soaked sample was filtered through cheese clothes. The residue was rinsed with more volume of methanol and filtrate was further filtered through filter paper to remove more of the solid particles. The filtrate was subjected to solvent recovery with the aid of rotary evaporator until dry/semi-dry residue was collected. This served as crude extract and was kept in the refrigerator awaiting to be used on the test organism.

Yield was calculated by the formula

$$\text{Yield of fraction (\%)} = \frac{\text{weight of fraction}}{\text{Total weight of sample}} \text{ x } 100$$

Test Organism

Trypanosoma brucei brucei was purchased from National Institute of Trypanosomiasis Research (NITR) Kaduna, Kaduna State, Nigeria. The organism was maintained in albino mice and kept in animal house, Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria by continuous passage into donor mice. The parasitemia level was monitored using the tail blood. The tail of the *T. brucei brucei* infected albino mice was sterilized using methylated spirit and the presence of the parasites was determined using light microscope at x 40 magnification by wet mount method of Murray et al, (1983). The degree of parasitemia was determined by rapid matching technique of Herbert and Lumasden (1976) as the values obtained was expressed in logarithmic figures.

Experimental animals

The albino mice needed for the work were purchased from the Department of Veterinary Pharmacology animal house Ahmadu Bello University, Zaria. The mice were kept in well ventilated plastic cages and were exposed to 12 hours light and dark and allowed free access to

grower feed and distilled water. The mice were allowed 7 days period of acclimatization before they were inoculated.
Ethical clearance was obtained from ABU Committee on Animal Use and Cure (ABUCAUC).

Inoculation of donor mice

Blood was collected in ethyldiaminetetraacetate coated syringe by cardiac puncture of a heavily infected albino mouse. The infected blood collected was diluted with normal saline. 0.1 ml of the diluted blood that contains 1 or 2 parasite per field (microscopic field) was inoculated into two clean healthy albino mice intraperitoneally to serve as the donor. Infection was monitored every morning by microscopic examination of blood sample obtained from the tail of the infected mice.

***In vitro* studies/Estimation of IC_{50}**

In vitro antitrypanosomal activity was carried out in triplicate in 96 well micro titre plates (Flow Laboratory Inc. Melean, Viginia 22101, USA). Twenty microlitre of blood containing about 40 – 60 parasites per field obtained from donor mouse was mixed with 5 µL of extract solution of 1µg/ml, 0.8µg/ml, 0.6µg/ml and 0.5µg/ml were prepared in 50 mM phosphate buffer saline glucose pH 7.2. A control was set up which contained the parasite suspended in phosphate buffer saline glucose only. A reference test was equally set up with 3.5mg concentrations of Diaminal^{R} (445 mg Diminazenediaceuturate + 555 mg Phenanzone/g, Eagle Chemical Company Ltd, Ikeja Nigeria) a commercial trypanocidal drug. The set up were incubated for 10 minutes at 37^{0}C.
After the 10 minutes of incubation 2 µl of the test mixture was placed on a separate microscope slide covered with cover slips and the parasites were observed every 20 minutes for a total duration of 120 minutes. Cessation or drop in parasites motility was taken as a measure of trypanocidal activity of extract treated blood compared to parasites loaded control blood without extract as described by Atawodi et al, (2003). The concentration that inhibits parasites population by 50 % (IC_{50}) was determined for extract by interpolation method of plotting the graph of % inhibition against concentration.

Results

Table 1. Showing percentage yield of crude extract of *O.gratissimum*

Plant	% yield
Ocimumgratissimum	6.72

Table 2. Showing *T. brucei brucei* parasite count of 20 µl infected blood incubated at 37^{0} C for 10 minutes with 5 µl *O. gratissimum* extract at varied concentration. Reading taking in duplicate.

Conc. (µg)	Average Parasite count/Time (minutes)							Percentage inhibition						
	10	20	40	60	80	100	120	10	20	40	60	80	100	120
Extract														
1000	34	26	16	11	6	3	1	32	48	68	78	88	94	98
800	36	30	27	20	16	11	7	28	40	46	60	68	78	86
600	37	34	29	24	19	15	10	26	38	42	52	62	70	80
500	41	36	31	27	22	18	13	18	28	38	46	56	64	74
Standard drug Diaminazene														
3500	10	3	-	-	-	-	-							
Buffer														
50mM	49	47	42	39	35	31	22							

Table 2. Showing IC_{50} of *Ocimum gratissimum* leaf methanol extract against *T. brucei brucei.* Value obtained from extrapolated plot of percentage inhibition against concentration in µg/ml.

Plant	**IC_{50} (µg/ml)**
Ocimumgratissimum	1.02±0.002

Discussion

The results show that *Ocimum gratissimum* plants obtained from Nigeria have antitrypanosomal activity as reflected in their IC_{50} of 1.02±0.002µg/ml.(table 3). This implies that geographical location did not affect the potentials of the plants' medicinal values in terms of their activity against trypanosomes. The estimated IC_{50} *O. gratissimum* differs in values with Abiodu et al, (2012) earlier report of IC_{50} 2.38±0.13 µg/ml, 2.08±0.01 µg/ml and 5.45±1.14 µg/ml for n-heaxane, ethyl acetate and methanol respectively for *O. gratissimum* extracts. Although, they only worked on the crude extract, our intention is to purify and characterize the active compound in this plant of interest. However, it is hopeful that as we go further in our purification process the efficacy of the plant sample shall be improved.

Conclusion

O. gratissimum sample gotten from our sample locations in Nigeria have antitrypanosomal activity against *T. brucei brucei*. The plant should be considered as an antitrypanosomal plant of ethnomedicinal values in Nigeria.

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